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# PRELIMINARY REPORT ON THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLEGHENIENSIS.<sup>1</sup>

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Although the eggs of the hellbender, *Cryptobranchus (Meno-poma) allegheniensis*, have been eagerly searched for by embryologists, a few unfertilized eggs only have hitherto been obtained. Aside from a brief description of an embryo in an advanced stage by McGregor ('96), there is no record of any observations on the development. The manner in which the eggs are fertilized has not been described.

This gap in our knowledge of comparative embryology is serious, particularly as very little is known about the development of the two other members of the family<sup>2</sup> Amphiumidæ: the giant salamander of Japan, *Cryptobranchus japonicus* (*Megalobatrachus maximus* Schlegel), and the American *Amphiuma means*.

During the early part of September, 1905, in northwestern Pennsylvania, I was so fortunate as to obtain fertilized eggs of *Cryptobranchus allegheniensis* in abundance, both from specimens in captivity and from the natural habitat of the animal.

## I. THE SEXUAL ELEMENTS.

A. *The Egg*. — The egg proper is perfectly spherical when fresh, about the size of a large pea, and bright yellow in color — a rather deep yellow at the lower pole, grading to a pale yellow at the upper. There is no black pigment such as is found in the eggs of most amphibians. A very thin transparent membrane, the vitelline membrane, quite inconspicuous in fresh material, closely invests the egg. Each egg, with its vitelline membrane, floats in a clear fluid within a hollow sphere or capsule about the size of a large grape, formed by the thick gelatinous outer

<sup>1</sup> Contributions from the Zoölogical Laboratory of the University of Michigan, No. 105.

<sup>2</sup> Gadow's classification is followed ('01).

envelope, the secretion of the oviduct. This outer egg envelope is produced at opposite poles of the capsule to form a slender cord connecting the eggs in a string (Fig. 1). The envelope is

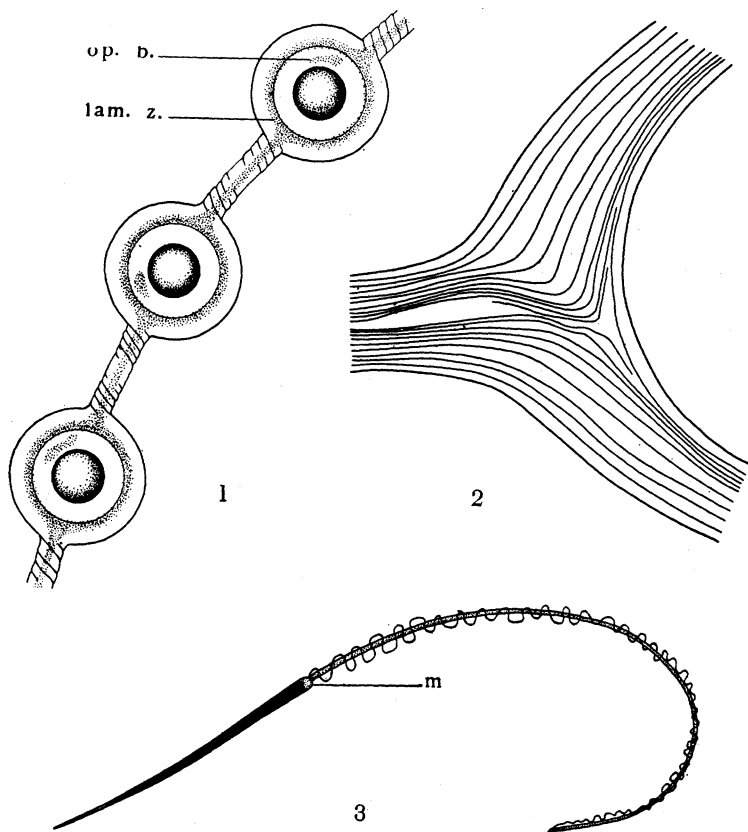


FIG. 1. Eggs and egg envelopes of *Cryptobranchus*, natural size. *op.b.*, opaque body; *lam.z.*, lamellar zone of envelope.

FIG. 2. Optical section (longitudinal) through lamellar zone of envelope in region of junction of egg capsule with connecting cord. Magnified about thirteen diameters.

FIG. 3. Spermatozoön, magnified about five hundred diameters. *m.*, middle piece.

perfectly transparent when fresh, except that wherever viewed tangentially its inner layers have a milky appearance, represented by the shaded zone in Fig. 1, and due to a fine lamellar structure sketched in optical section, with a magnification of about thirteen diameters, in Fig. 2. The misty appearance is

caused by the diffusion of light passing through these concentric layers tangential to their inner surfaces. The core or axis of the connecting cord also has the same milky appearance, due to a continuation of the lamellar structure.

The outer surface of the capsule and cord is, to ordinary observation, perfectly smooth and regularly rounded when the material is in fresh condition. But a careful examination, especially with the aid of a lens, reveals a delicate longitudinal striation due to fine parallel grooves or scratches separated by nearly equal intervals. This structure is too minute to be represented in Fig. 1. In some portions of the string the striæ have a slightly spiral direction about the axis of the cord and capsule. The striæ appear as they would if made by the scratching of the surface of the envelope against the teeth of a fine comb; perhaps they are caused by friction of the envelope against papillæ of some sort in the lower portion of the oviduct or in the cloaca, while the gelatinous material is yet soft.

After the eggs have been in water for several days, or after preservation in formalin or alcohol, the outer layer of the egg envelope becomes cast into conspicuous wavy folds or wrinkles of a different nature from the striæ noted above. These folds appear first at the ends of the connecting cord adjacent to the egg capsule and have here a strongly marked spiral arrangement, suggestive of the chalazæ of the hen's egg (see Fig. 1); later the folding may extend over the capsule and the middle of the cord, but seldom with so regular a spiral arrangement in these regions. Sometimes the spiral is continuous from one capsule to another. As a rule the spiral is constant in the direction in which it extends about the axis in all portions of the cord and capsule.

The inner layer of the lamellar core of the cord in some cases exhibits a marked twisted or spiral arrangement like that of the inner portion of the cord connecting the eggs of *Ichthyophis* as described by the Sarasins ('87-'93).

The following measurements were taken :

(1) Diameter of egg proper,	7 mm.
(2) " " " with envelopes,	18 "
(3) " " connecting cord,	5 "
(4) Distance from one egg to another, measured from center to center,	3 cm.

These figures represent the average of several measurements, the range of variation of the first dimension being small, of the others considerable, in eggs from the same parent. The egg proper ordinarily sinks to the bottom of the fluid which surrounds it, so that when viewed from above it is magnified by the lens-shaped capsule so as to appear considerably larger than is indicated by direct measurement.

Occasional strings of egg capsules are found without any eggs enclosed. These empty egg envelopes have the same structure as the others, and usually occur as a portion of a string of capsules the remainder of which contains eggs. A few of the empty egg capsules are double, formed by the union of two capsules without a connecting cord; in such cases the cavities of the two capsules are separated only by a very thin gelatinous septum.

In fertilized eggs in an early stage of development, preserved in formalin without removal from their envelopes, I found floating in the liquid between the egg and its envelope a fairly large irregular and slightly opaque mass, in appearance like a faint white cloud (see Fig. 1). This mass had not been noticed in the capsule of the living egg, probably because it was then transparent. Under the microscope it was found to consist of a clear gelatinous matrix in which were embedded numerous large round cells each with a very large nucleus and often with what appeared to be a large vacuole. By the use of a dissecting lens the individual cells could be seen while still within the envelope. In some egg capsules they occur diffused throughout the liquid of the cavity, not aggregated in a mass to form an opaque body. Numerous spermatozoa are present in the opaque body, but they also occur floating in the surrounding liquid and embedded in the egg capsule. Whether the opaque body is present in unfertilized eggs I have not at present the material to determine. The unfertilized eggs of *Cryptobranchus alleghehiensis* have been briefly described by Reese ('04) but without mention of any such feature as the opaque body described above. Both opaque body and spermatozoa regularly occur in egg capsules that do not contain eggs. The origin and function of the cells of the opaque body are at present problematical.

The eggs of *Cryptobranchus allegheniensis* bear a rather close resemblance to those of *C. japonicus* as described by Sasaki ('87), Kerbert ('04), Ishikawa ('04) and de Bussy ('05). Other amphibians whose eggs are fastened together like a string of beads are *Amphiuma* (Hay, '88 and '90), *Desmognathus*, *Ichthyophis*, *Hypogeophis* (Brauer, '97), and *Alytes*. The eggs of *Bufo* are arranged in strings but without the marked constriction of the envelope between the eggs characteristic of the other forms mentioned.

B. *The Sperm.* — The spermatozoön (Fig. 3) is of considerable size, about  $225\ \mu$  long, but with an unusually small middle-piece. The head of the spermatozoön, excepting the tip or acrosome, stains deeply with Conklin's modification of Delafield's hæmatoxylin. The head is very long and slender, tapering gradually to the extremely fine-pointed acrosome, enabling the spermatozoön to pierce easily through the vitelline membrane. The tail-piece is provided with an undulating membrane, bordered with a convoluted filament.

The spermatozoön of *Cryptobranchus allegheniensis* resembles that of *C. japonicus* as figured by Ishikawa ('04). It is also quite similar to the spermatozoön of *Amphiuma* (McGregor, '99), but the latter has a larger middle-piece and a slightly barbed acrosome.

## II. BREEDING HABITS.

A. *Method of Fertilization.* — A newly-captured female was isolated in an aquarium not previously occupied by any other specimen. A large number of eggs were laid, which contained spermatozoa within the egg capsule; moreover the subsequent development of these eggs proved them to have been fertilized. Hence there can be no doubt that in *Cryptobranchus allegheniensis*, as in all other Urodeles so far as known, fertilization, unlike that of the *Anura*, is internal. The mode of transference of the male element to the cloaca of the female has not been ascertained.

Nothing conclusive has been established concerning the method of fertilization of the nearest relative of *Cryptobranchus allegheniensis*, the giant salamander of Japan. In the case of *Amphiuma*, Davison ('94 and '95) believed a transference of spermatozoa

from male to female to take place by means of an apposition of the lips of the two cloacæ, basing this belief on what appears to me insufficient evidence; such a process is moreover not in harmony with known methods of fertilization among the Urodeles, in which fertilization is effected by means of spermatophores and without direct cloacal contact (Jordan, '91 and '93). Internal fertilization is said to occur in the Gymnophiona, *Ichthyophis* and *Hypogeophis*, as well as in the Urodeles.

B. *Breeding Season*. — Fertilized eggs were laid by a specimen in captivity on September 6, and another spawning by the same female took place during the night of September 7–8. Eggs in the first cleavage stage were found in the natural habitat of the animal on September 7, and another spawning of eggs in an advanced stage of segmentation was found in the same habitat on September 8.

Townsend ('82) records the laying of some eggs by a specimen in captivity during the month of August. McGregor ('99) states that the eggs are deposited in August and September.

No direct observations as to the time when the females are fertilized were obtained; but male specimens killed during the early part of September were found to have the vasa deferentia distended by a large quantity of seminal fluid containing an abundance of ripe spermatozoa. According to McGregor ('99) "the sexual union must occur very near or at the time of egg-laying, for the female is devoid of spermathecæ, and the spermatozoa do not ripen until late in August."

Eggs of *C. japonicus* were found by Sasaki ('87) in August. Kerbert ('04) records the spawning of a specimen in captivity during the night of September 18, 1902, and again on September 19, 1903. DeBussy ('05) reports that eggs were laid by Kerbert's specimen during September 14, 1904, and several days following. According to Ishikawa ('04) the eggs are laid principally during the latter half of August, but also in September. Hence the breeding season coincides with that of *C. allegheniensis*.

Eggs of *Amphiuma* in an advanced stage of development were found by Hay ('88 and '90) on September 1. Davison ('94 and '95) states that the eggs are deposited in August or September, but without giving the data upon which this statement is based.

He found a viscid substance containing spermatozoa exuding from the vent of a male specimen in May (April, according to his earlier paper), and concluded that this month is the natural season for fertilization.

The occurrence of the breeding season of *Cryptobranchus* in the fall is in marked contrast to the habits of nearly all other Urodeles, since they lay their eggs in the spring. While the significance of this unusual breeding season is not readily apparent, it is, in the case of *C. allegheniensis*, in at least one respect adaptive. The animal is an inhabitant of streams that during spring and early summer are subject to frequent and destructive freshets, which would probably be disastrous to the development of eggs like those of *Cryptobranchus*. During late summer and fall the streams are shallow and the water comparatively quiet; floods are of rare occurrence. These factors do not affect the other amphibians of the region in the same way, since they are inhabitants of ponds not seriously disturbed by floods, and on account of the more abundant rainfall better adapted for breeding-grounds in the spring than later in the year. There is every reason to believe that these climatic conditions have been of long duration. Probably the same conditions prevail with regard to *C. japonicus*, which is an inhabitant of mountain streams similar to those in which *C. allegheniensis* occurs. In this connection Professor Jacob Reighard informs me that the increase of spring freshets in Michigan during recent years, aided no doubt by other effects of lumbering operations, has nearly caused the extinction of the grayling, a fish that breeds in the spring, and was formerly abundant; the trout, which breeds in the fall, now thrives in the same streams. These facts indicate the selective value of the factors mentioned, and support the view taken with regard to *Cryptobranchus*.

*C. Breeding Habitat.* — Eggs of *Cryptobranchus* were found in shallow water in what had once been the main channel of a large stream, but through which now only a portion of the water, separated from the main channel by an island, flows. This old river channel is extremely rocky, with a considerable incline, so that the shallow water alternately forms pools and rapids. Judging from the number of specimens seen, the locality is a



favorite haunt of *Cryptobranchus*. Adult hellbenders were seen in other portions of the stream in deeper water, but in no other situations were eggs found.

The locality described above bears a strong resemblance to the habitat of *C. japonicus* as illustrated by Ishikawa ('04).

D. *Habits of Oviposition*.—Two separate spawnings of eggs were found in the natural habitat of the animal, besides eggs laid on two different occasions by a specimen in captivity. On account of differences in the method of disposal of the eggs, these spawnings are best described separately.

The eggs found on September 7 were lying in gently flowing water about 2–4 inches deep, on a gravelly and stony bottom, within a space about 6 feet in diameter nearly enclosed by some large rocks which projected a foot or more out of the water. The eggs were arranged in long festoon-like strings, scattered over an area of about 2 x 5 feet. In a few places the eggs were grouped in masses, but these masses might readily be resolved into strings. All the eggs present were included in a very few strings; one string contained 27 eggs. Evidently the eggs had not been disturbed since being laid. One hundred and ten eggs were counted in full view; but some other eggs had sunk down into crevices between and beneath stones; these brought the entire number up to 135. The eggs lay for the most part in direct sunshine, and the shallow running water furnished them with abundant aëration. No adult hellbenders were seen in the immediate vicinity.

The eggs were conspicuous because of their size and number, and were really beautiful objects, on account of the regularity of their form, the festoon-like manner of their arrangement on the pebbly bottom, the bright yellow of the yolk, and the perfect transparency of their gelatinous envelopes except where bordered with a delicate misty gray.

On September 8 another spawning of eggs, perhaps 50 in number, was found not far from the spot where eggs were discovered on September 7. The eggs were in water 3–5 inches deep, on the down-stream side of a rock, and most of them in a shallow cavity about 16 inches wide and extending back about 8 inches under the rock. From the appearance of the surround-

ing and underlying gravel this hollow looked as if it had been dug out by some animal. The eggs had been disturbed and scattered considerably; the strings were short, as if they had been much broken up, and many separated eggs were found. The eggs were thickly covered with silt. Both the envelopes and the contained eggs were, as a rule, slightly larger than those previously found. No adults were seen in the vicinity.

The eggs obtained on September 6 from a specimen in captivity were found rolled and tangled together in such an intricate manner that they seemed to occur in clusters or masses rather than in strings. The number was estimated at about 80, nearly all in one oblong mass. The solitary female did not appear to care for the eggs in any way.

On September 8 about 300 more eggs were laid by the same female specimen in captivity. The strings of eggs were aggregated in one large mass, but they were not so much tangled as in the case of those laid previously. There were present at the time in the same aquarium three male specimens, but so far as was observed, none of the adults paid any attention to the eggs. When all four specimens were killed a few days later for the purpose of determining the sex by dissection, the stomachs of the males were found distended with undigested eggs.

Upon examination, the female, which during captivity had laid nearly 400 eggs, was found to contain, at a rough estimate, seven or eight hundred more, in a state of development which indicated that had she been allowed to live they would all have been laid during the same season. Evidently the eggs are, in some cases at least, matured and laid in batches of a few hundred at a time.

According to Gadow ('01), *Amblystoma* alone among the Urodeles lays as many as 1,000 eggs in a single season. The facts stated above make it probable that *Cryptobranchus* rivals *Amblystoma* in the number of eggs laid. Kerbert's specimen of *C. japonicus* is reported to have laid 500 eggs in the fall of 1902, and about 900 in 1904. The eggs of *Amphiuma* found by Hay numbered about 150.

The absence of any evidence of brooding habits of either the male or the female *Cryptobranchus* is rather unexpected in view of the possession of brooding habits by closely related forms.

Concerning the nesting and brooding habits of *C. japonicus* Ishikawa says: "Das tier legt seine Eier in tiefe horizontal verlaufende Löcher, in denen das Wasser sehr ruhig ist. Manchmal ist solch ein Loch 10 oder mehr Fuss tief und kaum für das Licht zugänglich. Die Brutstellen für die Eier sind aber nicht immer so tief. Oft fand ich Eier in einem Loch nicht tiefer als 3 oder 4 Fuss. Oeffnet man ein solches Loch, so findet man eine abgerundete Stelle, deren Boden ganz rein gehalten ist. . . . Fast in jedem Loch, wo man von Ende August bis zu Anfang October ein weibliches Tier gefunden hat, findet man einen Eiklumpen. Dieser Umstand lässt schon vermuthen, dass das Tier eine Brutpflege hat wie *Ichthyophis* oder wie so viele andere Amphibien." Kerbert, however, asserts that it is the male that guards the eggs, and states that the sex of his specimens was carefully determined. The only external distinction between the sexes is that during the breeding season the lips of the cloaca of the male are greatly swollen. Ishikawa gives an illustration of an adult specimen of *C. japonicus* lying in a coil about a mass of eggs; Kerbert states of his specimen that the male, after driving away the female and also the small fishes present in the aquarium, crept between the folds of the mass of eggs, or sometimes simply lay down beside them, but in either case he kept the entire mass in motion by a pendulum-like movement of the entire body.

The eggs of *Amphiuma* found by Hay ('88 and '90) in an Arkansas swamp were in a comparatively dry situation, in a small excavation under a log several rods from the nearest water. The female was found coiled about the mass of eggs, protecting them and keeping them moist.

Other Amphibia for which brooding habits have been established are the Urodele, *Desmognathus*; the Gymnophiona, *Ichthyophis* and *Hypogeophis*; *Alytes* and several other Anura (Wiedersheim, '00). In the cases of *Desmognathus*, *Ichthyophis* and *Hypogeophis*, the female is said to care for the eggs; in the case of *Alytes*, the male.

*E. Adaptation of the Egg to its Environment.*—The egg proper is so soft and fragile that it can sustain only the most careful manipulation without injury; on account of its lack of firmness it soon becomes oblate from the effects of gravity. In view of

the fact that the eggs are laid in running water, the protection of such an egg from shocks and jars due to impacts against the rocks is an exceedingly important matter, and we find it accomplished by an admirable adaptation of the egg envelope. The tough but elastic egg capsule is inflated by osmosis with a liquid in which the egg freely floats. The turgid condition of the envelope increases its efficiency in protecting the egg. While ordinarily the egg sinks to the bottom of the surrounding liquid, and rests lightly upon the membrane beneath, there is so little friction between the egg and its envelopes that the latter may be rotated without turning the egg. A similar adaptation exists in the eggs of other amphibians, and in the eggs of teleosts (Reighard, '93); but it is particularly well shown in the egg of *Cryptobranchus*. The connecting cord is strong enough to support the weight of half a dozen eggs suspended out of water; it stretches greatly during the operation, but contracts to its usual length when the eggs are returned to the water. This elasticity of the connecting cord serves to deaden any shock to the eggs due to the tugging of the current or to the chain catching on to rocks while floating down stream.

The egg envelopes further serve to protect the eggs from little fishes that would otherwise devour them.

If eggs are left for several days undisturbed in the still water of a basin, some of them adhere to the envelopes beneath, interfering with the development. Probably under natural conditions the gentle agitation of the current tends to prevent this, performing for these eggs the same service afforded to the eggs of the hen by the mother when she turns them.

### III. SEGMENTATION.

The perfect transparency of the gelatinous envelopes of these eggs makes them very favorable material for watching the development of the living egg, since the process may be observed without removing the egg from the capsule. The large size of the eggs and the distinctness of the cleavage lines on their upper hemispheres also make them convenient objects for study. By using a mirror both poles of the egg may be watched at the same time, but observations on the lower hemisphere by this method

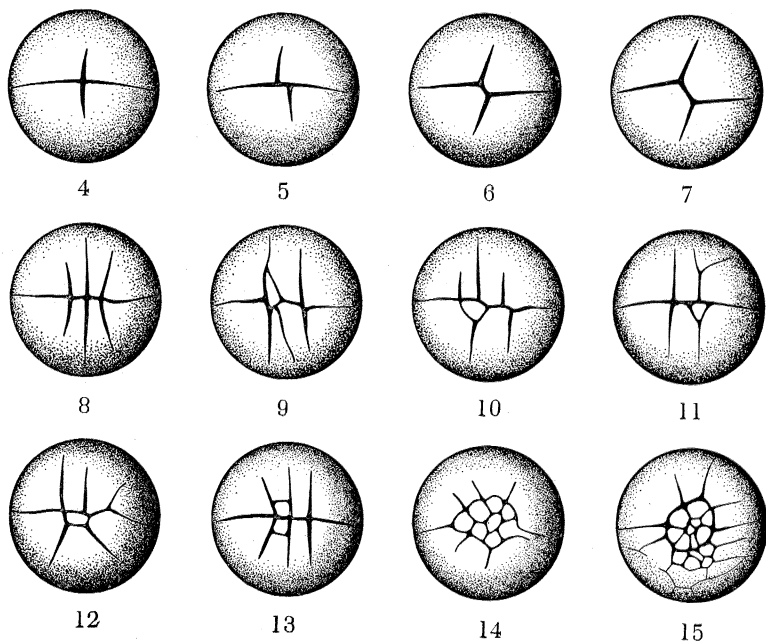
are rather difficult, on account of the faintness of the cleavage lines in this region. Material was preserved at intervals and camera drawings afterwards made of the principal stages ; but for the earliest stages the freehand sketches made from living material are sufficiently accurate, and have been used in preference to camera drawings from preserved material.

Some of the eggs found on September 7 were left to develop *in situ* as a check upon those studied under artificial conditions ; but so far as cleavage is concerned no difference could be detected between these eggs and those kept in a dish of shallow water.

The abundance of the material at hand made it possible to secure a series of drawings of the segmentation stages which should be fairly representative. The drawings include some of the most regular and geometrically perfect figures that could be found, and probably these may be regarded as typical ; but in *Cryptobranchus* as in other amphibians the amount of variation is great. Since departures from the type are not necessarily the result of pathological conditions, they should not be dismissed as abnormalities ; for they may be the expression of opposing factors in the development of the egg. Such factors are the proportion of yolk to protoplasm, the qualitative composition of the yolk determining the extent to which it responds to the sorting influence of gravity, and hereditary factors in the protoplasm ; variations in these factors affect the manner of cleavage, and according as one tendency or another prevails we may find the typical form or variations from it in definite directions. Hence the figures include representations of eggs in the same stage showing differences in the method of cleavage. There are of course individual differences in the rate as well as in the manner of cleavage.

A. *The First Cleavage Furrow.* — In eggs laid September 6 at about 6:30 P. M., there was so much diversity in the time of appearance of the first cleavage furrow that it is difficult to assign limits. Probably more cases of first cleavage were noticed at about 10 A. M. of the next day — 15 hours after the eggs were laid — than at any other time. In eggs laid during the night of September 7–8 the first cleavage furrows appeared in several eggs at about 5 P. M. of the next day, and cases were numerous at 6:30 P. M. The eggs found on September 7 at 10 A. M. were

in the first cleavage stage; observations on the rate of deposition of silt on their envelopes indicated that they had been laid during the preceding night or late in the afternoon of the day before.



FIGS. 4-15. Early segmentation stages of eggs of *Cryptobranchus*. Freehand sketches from living material magnified about  $\frac{1}{4}$  diameters.

FIG. 4. Egg found September 7 at 10 A. M., sketched at 1 P. M. The first cleavage line extends over exactly half a circumference.

FIGS. 5-7 show variations in the second cleavage. Fig. 5, egg laid September 6 at about 6:30 P. M., sketched September 7 at 3 P. M. Figs. 6 and 7, eggs found September 7 at 10 A. M., sketched at 1:30 P. M.

FIG. 8. Egg laid during night of September 7-8, sketched September 9 at 5:30 A. M.

FIGS. 9-11. Variations in third cleavage. Eggs laid during night of September 7-8, sketched September 9; Fig. 9 at 3:50 A. M., Fig. 10 at 2:30 A. M., Fig. 11 at 5:30 A. M.

FIG. 12. Egg laid during night of September 7-8, sketched September 9 at 5:45 A. M.

FIGS. 13 and 14. Fourth cleavage stages. Fig. 13, a later stage of egg represented in Fig. 8, sketched September 9 at 8 A. M.; Fig. 14, a later stage of egg represented in Fig. 9, sketched September 9 at 8 A. M.

FIG. 15. Egg found September 7 at 10 A. M., sketched September 8 at 8:45 A. M. Four nearly equidistant cleavage lines reach almost to the center of the lower pole.

It is not known whether the eggs of a given spawning are all fertilized at the same time, hence some of the diversity in the time of appearance of the first cleavage furrow may be due to a difference in the time of fertilization.

The first cleavage furrow begins as a pit, which gradually elongates, rapidly at first then more slowly. The point of origin of the first cleavage furrow remains always uppermost, even though the envelope is rotated.

*B. The Second Cleavage Furrow.*—This furrow makes its appearance about six hours after the first, which by this time has extended over about half the distance to the equator of the egg. The second cleavage furrow usually cuts the first at right angles, but some variations from this procedure are shown in the figures. (See Figs. 4-7.)

Some time before the appearance of the second groove, the first one appears constricted and narrow in the middle portion, while still broad at the ends. The earliest indication of the second furrow is usually a roughness in the region where the second groove is to intersect the first. The appearance of "Faltenkränzen" — a quivering of the surface with the formation of fine radiating or parallel wrinkles, which extend outward from the cleavage furrow for a moment, tremble, and disappear — a common phenomenon in the cleavage of the amphibian egg, is quite marked at the time of the beginning of the second cleavage furrow. For some time after its appearance the second furrow is much broader, though of course shallower, than the first.

*C. The Third Cleavage Furrow* (see Figs. 8-12). — These furrows mark the establishment of the 8-cell stage about four or five hours after the appearance of the second cleavage groove. Hence the third division is more rapid than the second. At the time when the third furrows are initiated the first furrow has usually reached or passed the equator; the second one is confined to the upper hemisphere.

The third cleavage furrows ordinarily begin as two pits in the first furrow, not far from its point of intersection with the second — the center of the animal pole of the egg — and equidistant from that point. From these pits the third furrows proceed in an approximately vertical direction. The third cleavage furrow seldom

originates from the center of the first furrow, and seldom reaches the lower pole, but extending obliquely in the lower hemisphere joins the second furrow at some distance from the pole (see plate, Fig. 16*B*, line *b*; Fig. 17*B*, lines *b*, *g* and *e*; Fig. 18*B*, lines *b*, *i* and *e*, etc.). Hence the third cleavage furrow is *intermediate between a true meridional cleavage and a latitudinal one*, approaching more nearly the meridional type. This will be made clearer by supposing the point of origin of the third cleavage furrow to be shifted along the first further from the animal pole; and its point of junction with the second to be shifted further from the lower pole; the third groove will then become latitudinal. On the other hand, if the two ends were shifted in the reverse directions, the cleavage lines would become truly meridional. Variations from the customary mode of cleavage tend to confirm the view that it is an intermediate or transitional form; while some third cleavage furrows originate at the center of the animal pole, giving a true meridional cleavage, others come in latitudinally. (See Figs. 9–12.)

D. *The Fourth Cleavage Furrow* (Figs. 13–15).—This comes in about 3 hours later—a briefer interval than that preceding the appearance of the third cleavage furrow. In its typical condition it is parallel to the equator, but close to the animal pole, so that the division is very unequal. Irregularities in this cleavage are very numerous.

E. *Later Segmentation Stages* (see plate, Figs. 16–24).—The later divisions occur with increasing rapidity, but with such irregularity that no definite arrangement of cleavage furrows can be made out. Cleavage proceeds rapidly near the animal pole, much more slowly in the equatorial region, and is greatly retarded in the lower hemisphere. At some time during the second day after the egg is laid the first cleavage furrow reaches the lower pole. At this time the upper hemisphere is cut up into a considerable number of cells. The macromeres continue to be very much larger than the micromeres. While so far as can be determined from a surface study of the egg, the cleavage is undoubtedly holoblastic, a strong tendency toward the meroblastic condition is evident.



Two days after the egg is laid, cell division in the upper hemisphere has advanced until the individual cells can no longer be seen with the naked eye. The macromeres are in general still quite large, but in several instances there was noted a greater multiplication of cells about the lower pole than in the equatorial region (see Fig. 24*B*).

F. *Comparison with Other Forms.* — De Bussy ('05) has described the principal segmentation stages of the eggs of *C. japonicus*. His material lacked eggs in the 2-cell and 4-cell condition; but beginning with the 8-cell stage the later cleavage stages are described and illustrated. A comparison with my own sketches of the segmentation of the eggs of *Cryptobranchus allegheniensis*, made before I knew of the existence of de Bussy's paper on *C. japonicus*, shows, as might be expected from the close relationship of the two forms, a marked similarity in the mode of cleavage. Since de Bussy's paper includes a comparison of the eggs of *C. japonicus* with those of other forms, a similar discussion for *C. allegheniensis* at the present time seems unnecessary. It should be added, however, that the segmentation of the eggs of *Desmognathus*, described by Wilder ('04) and Hilton ('04) also bears a considerable resemblance to that of *Cryptobranchus*.

I take pleasure in acknowledging my indebtedness for encouragement and advice to Prof. Jacob Reighard, at whose suggestion I first looked for the eggs of *Cryptobranchus*, and to Dr. O. C. Glaser, under whose direction the work was carried on after I returned to the university.

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## EXPLANATION OF PLATE.

All the figures are camera drawings from preserved material. Figs. 16-19 are magnified about three diameters, the remaining figures about four diameters. *A* and *B* indicate upper and lower hemispheres respectively. The small letters serve to identify lines which cross the equator.

FIGS. 16-18. Eggs laid during night of September 7-8, preserved September 9 at 2 P. M.

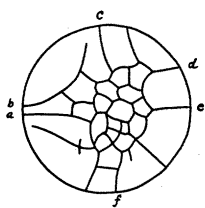
FIGS. 19 AND 20. Eggs found September 7 at 10 A. M., preserved September 8 at 9 A. M.

FIG. 21. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M. Lateral view.

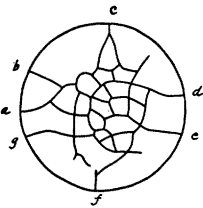
FIG. 22. Egg laid during night of September 7-8, preserved September 11 at 10 A. M.

FIG. 23. Egg found September 7 at 10 A. M., preserved September 10 at 8 A. M.

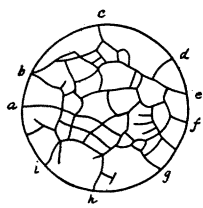
FIG. 24. Egg laid September 6 at 6:30 P. M., preserved September 8 at 8:30 A. M.



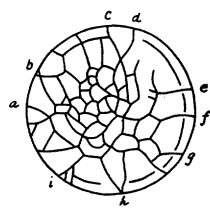
16A



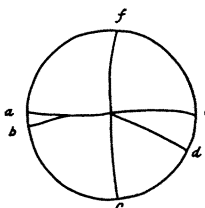
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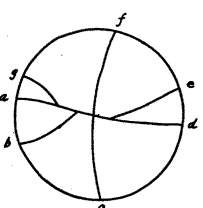
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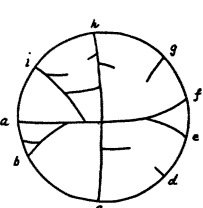
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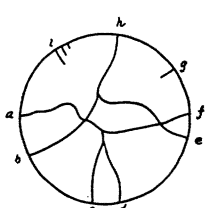
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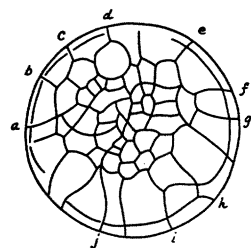
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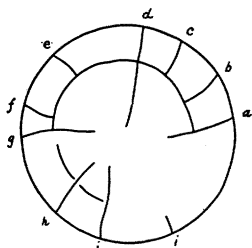
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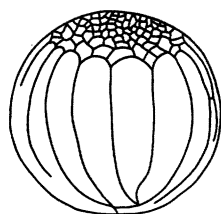
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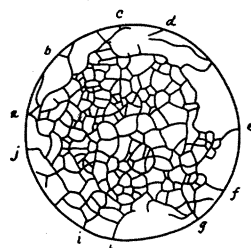
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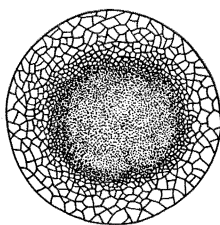
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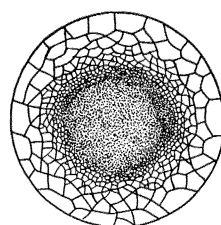
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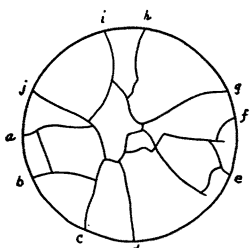
22A



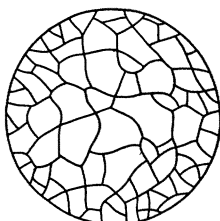
23A



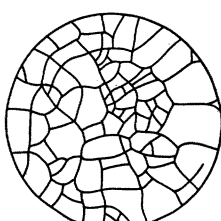
24A



22B



23B



24B